

Figure 1. N-linked oligosaccharides for glycosylation

Peptide sequence (SEQ ID NO:5):

```
MPFVNKQFNY KDPVNGVDIA YIKIPNAGQM QPVKAFKIHN KIWVIPERDT FTNP EEGDLN
PPPEAKQVPV SYYDSTYLST DNEKDNYLKG VTKLFERIYS TDLGRMLLTS IVRGIPFWGG
STIDTELKVI DTNCINVIQP DGSYRSEELN LVIIGPSADI IQFECKSFGH EVLNLTRNGY
GSTQYIRFSP DFTFGFEESL EVDTNPLLGA GK FATDPAVT LAHEL IHAGH RLYGIAINPN
RVFKVNTNAY YEMSGLEV SF EELRTFGGHD AKFIDSLQEN EFRLYYYNKF KDIAS TLNKA
KSI VGT TASL QYMKNVFKEK YLLSEDTSGK FSVDK LKFDK LYKMLTEIYT EDNFVKFFKV
LNRKTYLNFD KAVFKINIVP KVN YTIYDGF NLRNTNLAAN FNGQNT EINN MNFTKLKNFT
GLFEFYKLLC VRGIITSKTK SLDKGYNKAL NDLCIKVNNW DLFFSP SEDN FTNDLNKGEE
ITS D TNIEAA EENISLDLIQ QYYLTFNFDN EPENISIENL SSDIIGQLEL MPNIERFPNG
KKYELDKYTM FHYLRAQEFE HGKSRIALT N SVNEALLNPS RVTFFSSDY VKKVNKATEA
AMFLGWVEQL VYDFTDETSE VSTTDKIADI TIIIPYIGPA LNIGNMLYKD DFGALIFSG
AVILLEFIPE IAIPVLGTFA LVS YIANKVL TVQTIDNALS KRNEKWDEVY KYIVTNWLAK
VNTQIDLIRK KMKEALENQA EATKAIINYQ YNQYTEEEKN NINFNIDDL S SKLNESINKA
MININKFLNQ CSVSYLMNSM IPYGVK RLED FDASLKDALL KYIYDNRGTL IGQVDR LKDK
VNNTLSTDIP FQLSKYVDNQ RLLSTFTEYI KNIINTSILN LRYESNHLID LSR YASKINI
GSKVNFDPID KNQIQLFNLE SSKIEVILKN AIVYNSMYEN FSTSFWIRIP KYFNSISLNN
EYTIINC MEN NSGWK VSLNY GEI IWT LQDT QEIKQRV VFK YSQMINISDY INRWIFVTIT
NNRLNNSKIY INGR LIDQKP ISNLGNIHAS NNIMFKLDGC RDTHRYIWIK YFNLFDKELN
EKEIKDLYDN QSNSGILKDF WGDYLQYDKP YYMLNLYDPN KYVDVNNVGI RGYMYLKGPR
GSMVTTNIYL NSSLYRGTKF I IKKYASGNK DNIVRNNDRV YINVVVKNKE YRLATNASQA
GVEKILSALE IPDVGNLSQV VVMKSKNDQG ITNKCKMNLQ DNNGNDIGFI GFHQFN NIAK
LVASNWYNRQ IERSSRTLGC SWEFIPVDDG WGERPL
```

Peptides containing the motif 'N-X-S/T/C (X not P)' (underlined):

position	Peptide	SEQ ID NO:
167-177	SFGHEVLN <u>L</u> TR	6
382-393	VN <u>Y</u> TIYDGFNLR	7
394-415	NTNLAANFNGQNT EINN <u>M</u> NF TK	8
418-427	<u>N</u> FTGLFEFYK	9
457-477	VNNWDLFFSP SEDN <u>N</u> FTNDLN K	10
478-536	GEEITS D TNIEAAEENISLD LIQ QYYLTFNFDNEP <u>E</u> NI SI	11
773-779	ENLSSDIIGQLELMPNIER	12
773-779	LNESINK	12
787-806	FLNQCSVS YLMNSMIPYGVK	13
841-855	VNNTLSTDIPFQLSK	14
872-882	NIINTSILNLR	15
930-948	NAIVYNSMYENFSTSFWIR	16
952-975	YFNSISLNN EYTIINC MENN SGWK	17
1001-1013	YSQMINISDYINR	18
1024-1028	LNN <u>S</u> K	19
1086-1098	DLYDNQSN SGILK	20
1141-1156	GSMVTTNIYLNSSLYR	21
1193-1204	LATNASQAGVEK	22
1205-1224	ILSALEIPDVGNLSQVVVMK	23

Figure 1 Continue.

Peptide sequence (SEQ ID NO: 24):

KTKSLDKGYN KALNDLCIKV NNWDLFFSPS EDNFTNDLNK GEEITSDTNI EAAEENISLD
 LIQQYYLTFN FDNEPENISI ENLSSDIIGQ LELMPNIERF PNGKKYELDK YTMFHYLRAQ
 EFEHGKSRIA LTNSVNEALL NPSRVYTFFS SDYVKKVNKA TEAAMFLGWV EQLVYDFTDE
 TSEVSTTDKI ADITIIIPYI GPALNIGNML YKDDFVGALI FSGAVILLEF IPEIAIPVLG
 TFALVSYIAN KVLTVQTIDN ALSKRNEKWD EVYKYIVTNW LAKVNTQIDL IRKKMKEALE
 NQAEATKAI NYQYNQYTEE EKNNINFNID DLSSKLNESI NKAMININKF LNQCSVSYLM
 NSMIPYGVKR LEDFDASLKD ALLKYIYDNR GTLIGQVDRL KDKVNNTLST DIPFQLSKYV
 DNQRLSTFT EYIKNIINTS ILNLRYESNH LIDLSRYASK INIGSKVNFD PIDKNQIQLF
 NLESSKIEVI LKNAIVYNSM YENFSTSFWI RIPKYFNSIS LNNEYTIINC MENNSGWKVS
 LNYGEIIWTL QDTQEIQRV VFKYSQMINI SDYINRWIFV TITNNRLNNS KIYINGRLID
 QKPISNLGNI HASNNIMFKL DGCRDTHRYI WIKYFNLFDK ELNEKEIKDL YDNQNSNGIL
 KDFWGDYLYQ DKPYMLNLY DPNKYVDVNN VGIRGYMYLK GPRGSVMTTN IYLNSSLYRG
 TKFIIKKYAS GNKDNIVRNN DRVYINVVVK NKEYRLATNA SQAGVEKILS ALEIPDVGNL
 SQVVMKSKN DQGITNKCKM NLQDNNGNDI GFIFGHQFNN IAKLVASNWY NRQIERSSRT
 LGCSWEFIPV DDGWERPL

Peptides containing the motif 'N-X-S/T/C (X not P)':

position	peptide	SEQ ID NO:
20-40	VNNWDLFFSPSEDNFTNDLN K	25
41-99	GEEITSDTNIEAAEENISLD LIQQYYLTFNFDNEPENISI ENLSSDIIGQLELMPNIER	26
336-342	LNESINK	27
350-369	FLNQCSVSYLMNSMIPYGVK	28
404-418	VNNTLSTDIPFQLSK	29
435-445	NIINTSILNLR	30
493-511	NAIVYNSMYENFSTSFWIR	31
515-538	YFNSISLNNEYTIINCMENN SGWK	32
564-576	YSQMINISDYINR	33
587-591	LNNSK	34
649-661	DLYDNQNSNGILK	35
704-719	GSVMTTNIYLNSSLYR	36
756-767	LATNASQAGVEK	37
768-787	ILSALEIPDVGNLSQVVMK	38

Figure 2.

Peptide sequence (SEQ ID NO: 39):

MPFVNKQFNY KDPVNGVDIA YIKIPNAGQM QPVKAFKIHN KIWVIPERDT FTNPEEGDLN
PPPEAKQVPV SYDSTYLST DNEKONYLKG VTKLFERIYS TDLGRMLLTS IVRGIPFWGG
STIDTELKVI DTNCINVIQP DGSYRSEELN LVIIGPSADI IQFECKSFGEH EVLNLTRNGY
GSTQYIRFSP DFTFGFEESL EVDTNPLLGA GKFDTPAVT LAHELIIHAGH RLYGIAINPN
RVFKVNTNAY YEMSGLEVSE EELRTFGGHD AKFIDSLQEN EFRLYYYNKF KDIASLTNKA
KSIVGTTASL QYMKNVFKEK YLLSEDTSKG FSVDKLKFDK LYKMLTEIYT EDNFVKFFKV
LNRKTYLNFD KAVFKINIVP KVNITYIDGF NLRNTNLAAN FNGQNTTEINN MNFTKLKNFT
GLFEFYKLLC VRGIITSKTK SLDKGYNKAL NDLCIKVNNW DLFFSPSEDN FTNDLNKGEE
ITSNTNIEAA EENISLDLIQ QYYLTFNFDN EPENISIENL SSDIIGQLEL MPNIERFPNG
KKYELDKYTM FHYLRAQEFH HGKSRIALTN SVNEALLNPS RVTTFSSDY VKKVNKATEA
AMFLGWVEQL VYDFTDETSE VSTDKIADI TIIIPYIGPA LNIGNMPLYKDFVGFALIFSG
AVILLEFIPE IAIPVLGTFA LVSYIANKVL TVQTIDNALS KRNEKWDEVY KYIVTNWLAK
VNTQIDLIRK KMKEALENQA EATKAIINYQ YNQYTEEEKN NINFNIDDL SCLNESINKA
MININKFLNQ CSVSYLMNSM IPYGVKRLD FDASLKDALL KYIYDNRGTL IGQVDRKDK
VNNTLSTDIP FQLSKYVDNQ RLLSTFTEYI KNIINTSILN LRYESNHLID LSRYSKINI
GSKVNFDPID KNQIQLFNLE SSKIEVILKN AIVYNSMYEN FSTSFWRIP KYFNSISLNN
EYTIINC MEN NSGWKVS LNY GEI IWT LQDT QEIKQRVVFK YSQMINISDY INRWIFVTIT
NNRLNNSKIY INGR LIDQKP ISNLGNIHAS NNIMFKLDGC RDTHRYIWI K YFNLFDKELN
EKEIKDLYDN QSN SGILKDF WGDYLQYDKP YYMLNLYDPN KYVDVNNVGI RGYMYLKGPR
GSVMTTNIYL NSSLYRGTKF I IKKYASGNK DNIVRNNDRV YINVVVKNKE YRLATNASQA
GVEKILSALE IPDVGNLSQV VVMKSKNDQG ITNKCKMNLQ DNNGNDIGFI GFHQFNNAK
LVASNWNRY IERSSRTLGC SWEFIPVDDG WGERPL

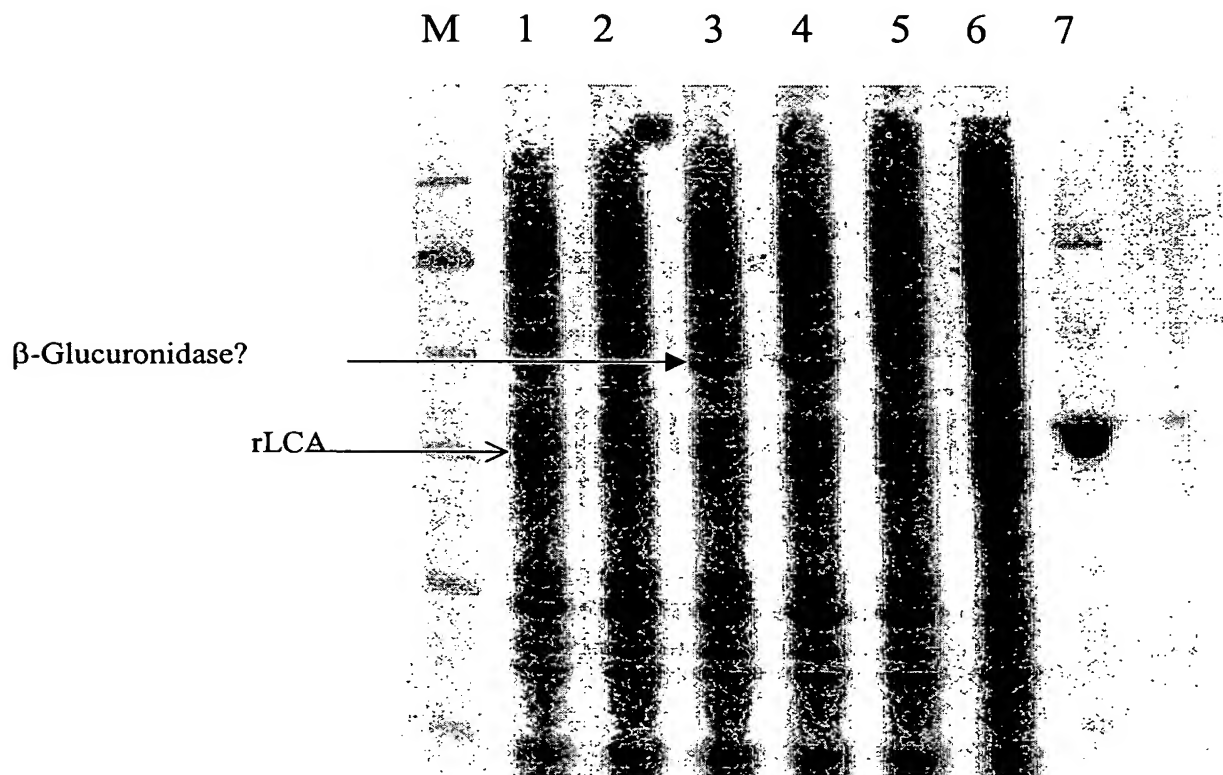
Peptides containing S or T (underlined):

position	peptide	SEQ ID NO:
49-66	DIF <u>TNPEEGDLNPPPEAK</u>	40
67-84	QVPV <u>SYYDSTYLSTDNEK</u>	41
90-93	G <u>VTK</u>	42
98-105	IY <u>STD</u> LGR	43
106-113	MLL <u>TS</u> IVR	44
114-128	GIPFWGG <u>STIDTELK</u>	45
129-145	VIDTNCINVIQPDGSYR	46
146-166	<u>SEELNLVIIGPSADIIQFEC</u> K	47
167-177	<u>SFGHEVLNLTR</u>	48
178-187	NGY <u>GSTQYIR</u>	49
188-212	FSPDFTFGFEES <u>LEV</u> DTNPL LGAGK	50
213-231	FATDPAVTLAHELIIHAGHR	51
245-264	VNTNAYYEM <u>S</u> GLEVS <u>F</u> EELR	52
265-272	<u>T</u> FGGHDAK	53
273-283	FIDSLQENEFR	54
292-299	DIAS <u>T</u> LNK	55
302-314	<u>SIVGTTASLQYMK</u>	56
321-330	YLL <u>S</u> EDTSKG	57
331-335	FSV <u>D</u> K	58
344-356	ML <u>T</u> EIYTEDNFVK	59
365-371	<u>T</u> YLNFDK	70
382-393	VNYTIIYDGFNLR	71
394-415	N <u>T</u> NLAANFNGQNTTEINNMNF <u>TK</u>	72
418-427	NFTGLFEFYK	73
433-438	GI <u>T</u> SK	74
439-440	<u>T</u> K	75
441-444	<u>S</u> LDK	76

Figure 2
Continue

457-477	VNNWDLFFSPSEDNFTNDLN K	77
478-536	GEEITSDTNIEAAEENISLD LIQYYLT FNFDNEPENISI ENLSSDIIGQLELMPNIER	78
548-555	YTMFHYLR	79
564-565	SR	80
566-581	IALTNSVNEALLNPSR	81
582-592	VYTFFSDDYVK	82
597-626	ATEAAMFLGWVEQLVYDFTD ETSEVSTTDK	83
627-649	IADITIIPYIGPALNIGNM LYK	84
650-688	DDFVGALIFSGAVILLEFIP EIAIPVLGTFALVSYIANK	85
689-701	VLT TVQTIDNALS K	86
712-720	YIVTNWLAK	87
721-729	VNTQIDLR	88
734-744	EALNQAEATK	89
745-759	AIINYQYNQYTEEEK	90
760-772	NNINFNIDDLSSK	91
773-779	LNESINK	92
787-806	FLNQCSVSYLMNSMIPYGVK	93
808-816	LEDFDA SK	94
828-836	GTLIGQVDR	95
841-855	VNNTLST DIPQLSK	96
862-871	LLSTFTEYIK	97
872-882	NIINTSILNLR	98
883-893	YESNHLIDL SR	99
894-897	YASK	100
898-903	INIGSK	101
912-923	NQIQLFNLESSK	102
930-948	NAIVYNSMYENFSTSFWR	103
952-975	YFNSISLNNEYTIINCMENN SGWK	104
976-994	VSLNYGEIHWTLQDTQEIK	105
1001-1013	YSQMINISDYINR	106
1014-1023	WIFVTITNNR	107
1024-1028	LNN SK	108
1035-1056	LIDQKPI SNLGNIHASNNIM FK	109
1062-1065	DTHR	110
1086-1098	DLYDNQSN SGILK	111
1141-1156	GSVMTTNIYLNSSLYR	112
1157-1159	GTK	113
1165-1170	YASGNK	114
1193-1204	LATNASQAGVEK	115
1205-1224	ILSALEIPDVGNLSQVVVMK	116
1225-1226	SK	117
1227-1234	NDQGITNK	118
1261-1269	LVASN WYNR	119
1274-1276	SSR	120
1277-1296	TLGCSWEFIPVDDGWGERPL	121

Figure 3



- M: See Blue Plus2 marker
1. pBAC-1/LCA, H227Y
2. pBAC-1/LCA
3. pBACgus-1/LCA, H227Y
4. pBACgus-1/LCA
5. AcNPV, negative control
6. Sf9 insect cells only
7. E.coli expressed LCA

Figure 4

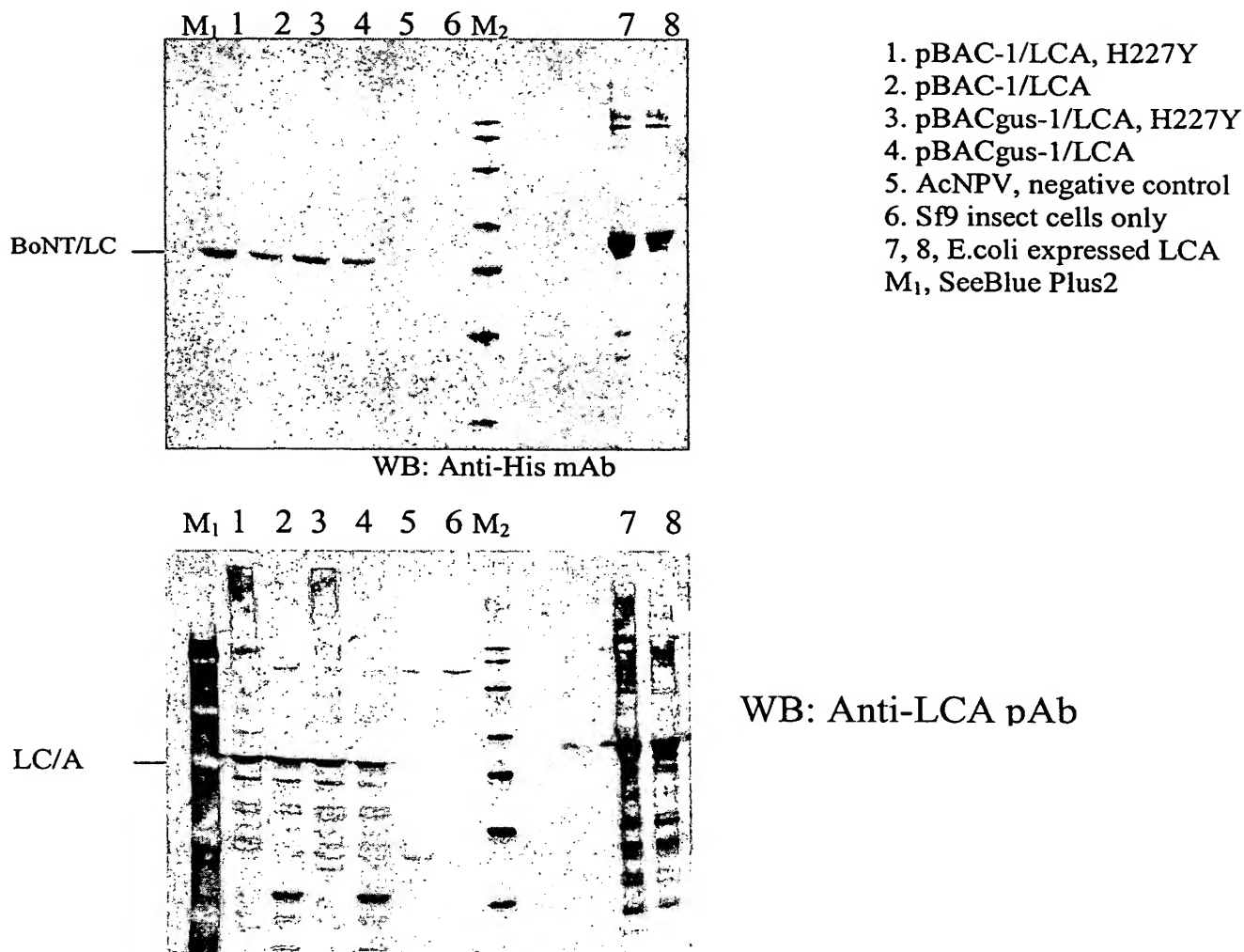
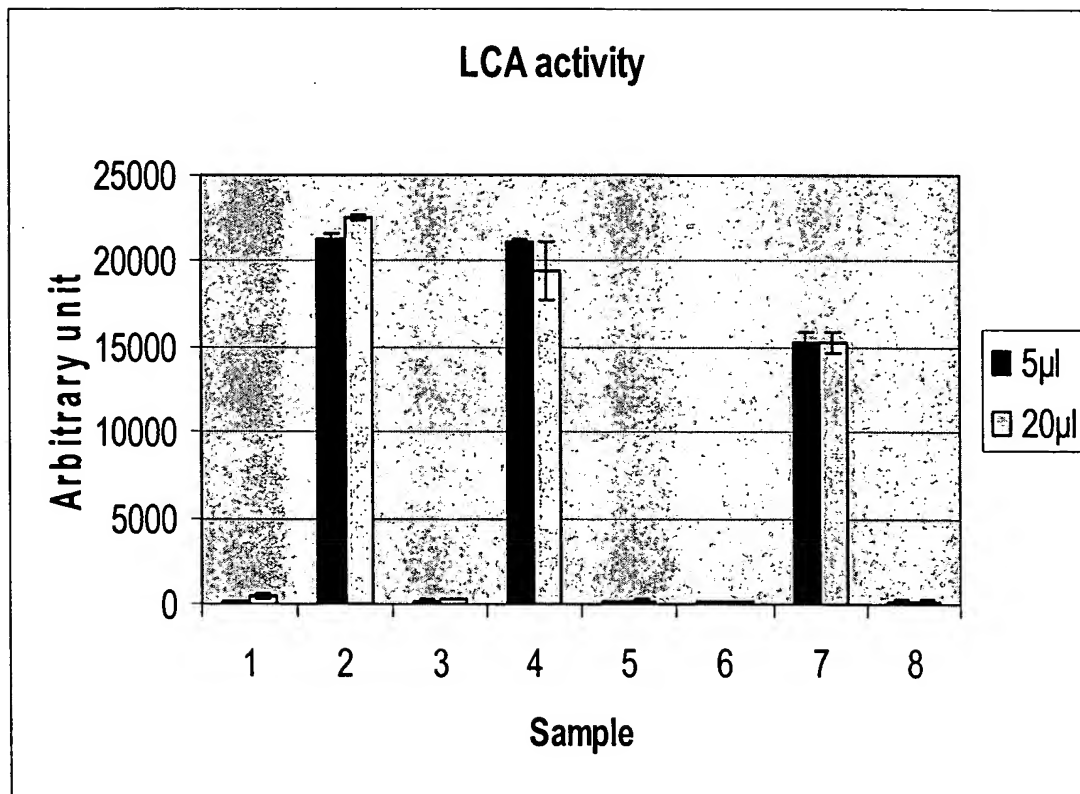


Figure 5



1. pBAC-1/LCA, inactive (H227Y)
2. pBAC-1/LCA, active
3. pBACgus-1/LCA, inactive (H227Y)
4. pBACgus-1/LCA
5. AcNPV, negative control
6. Sf9 insect cell lysate only
7. rLCA, positive control
8. Substrate only

Figure 6

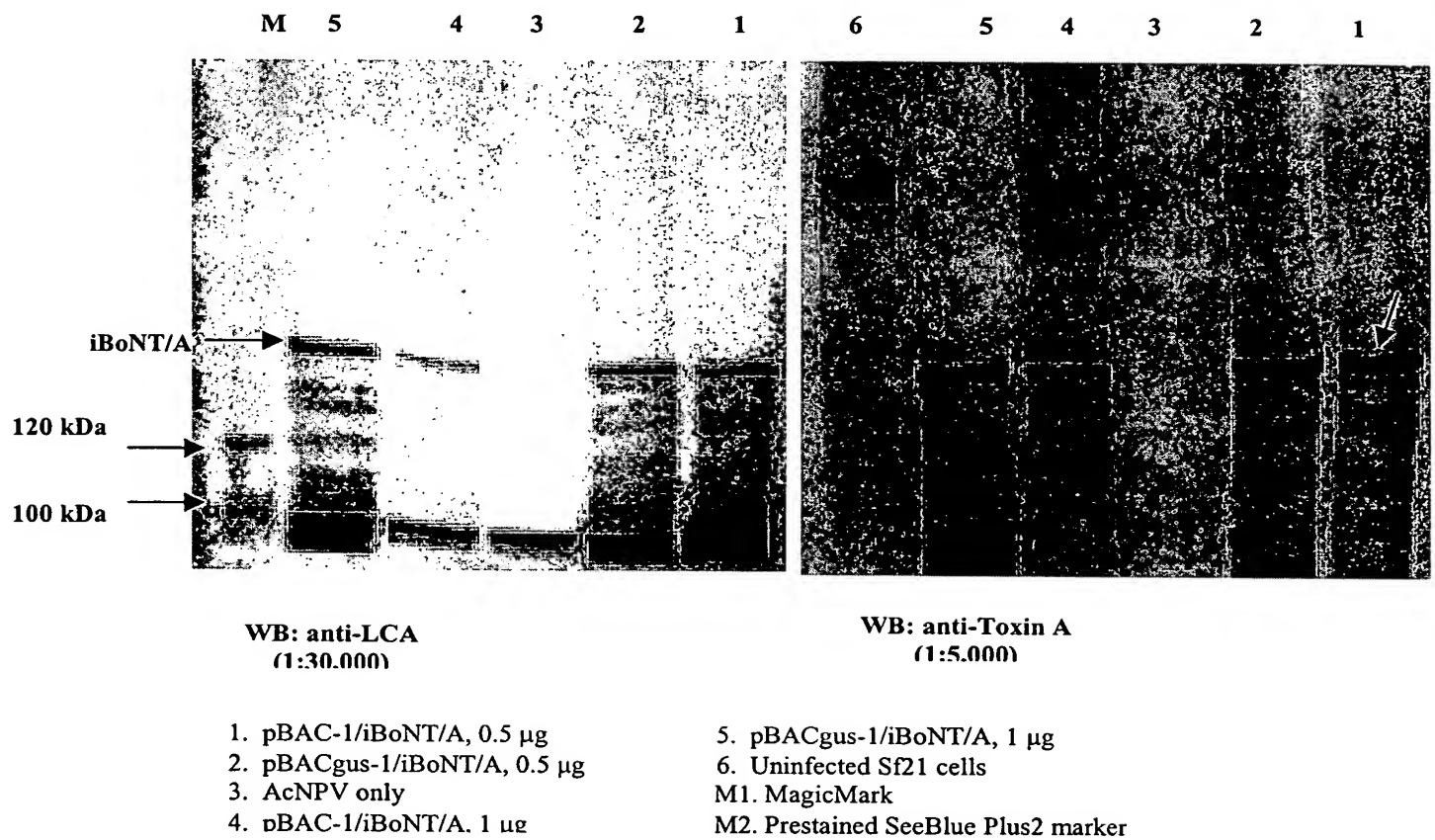


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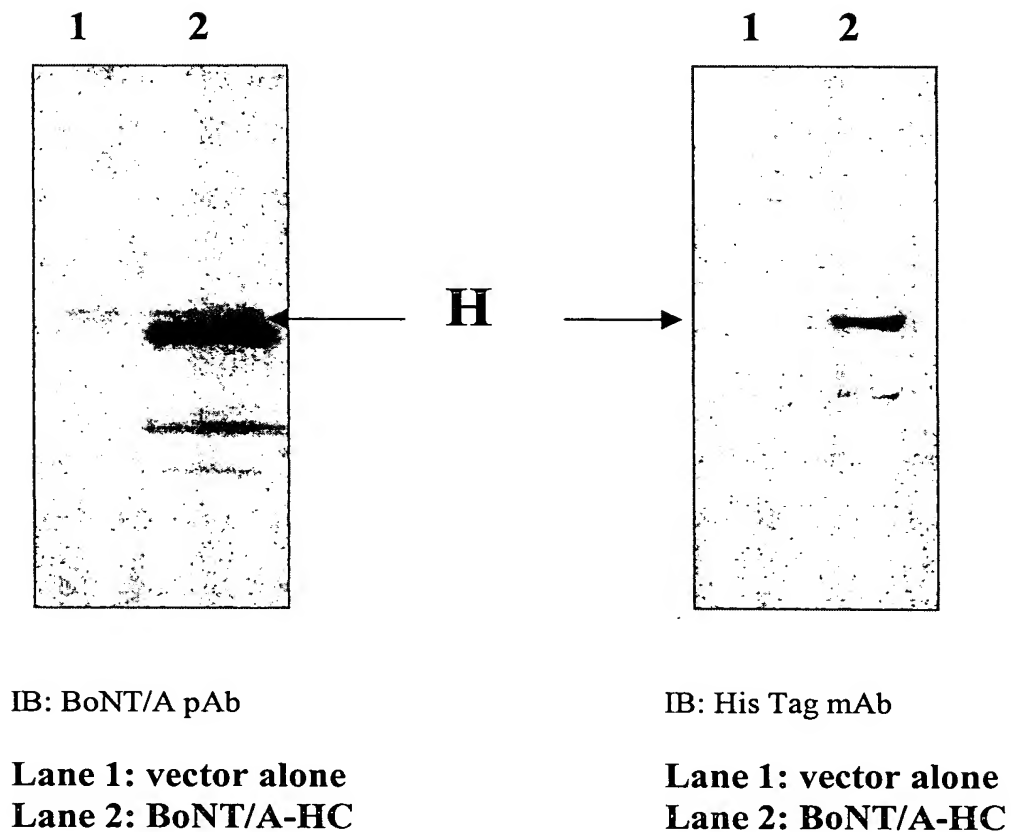


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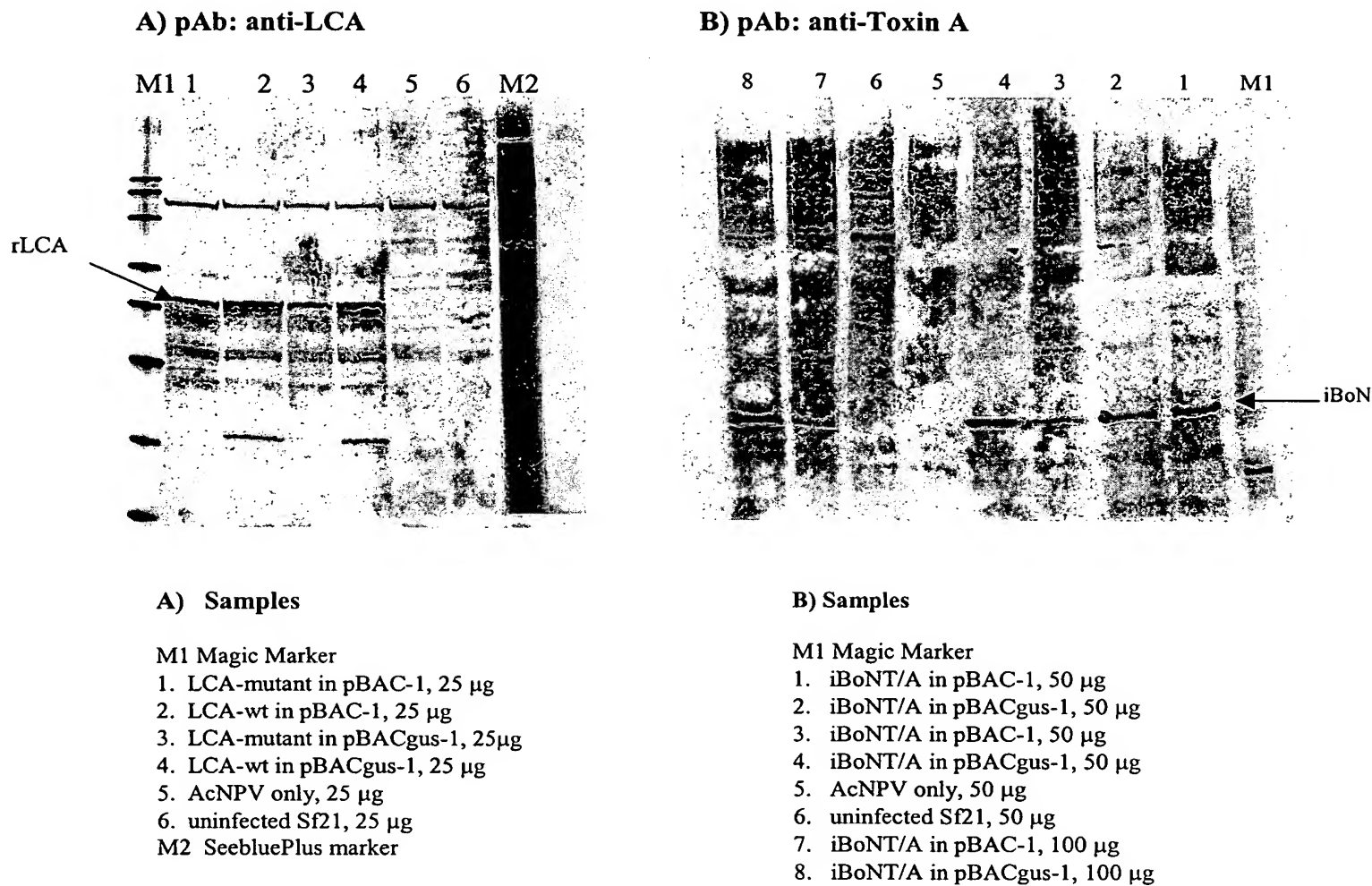
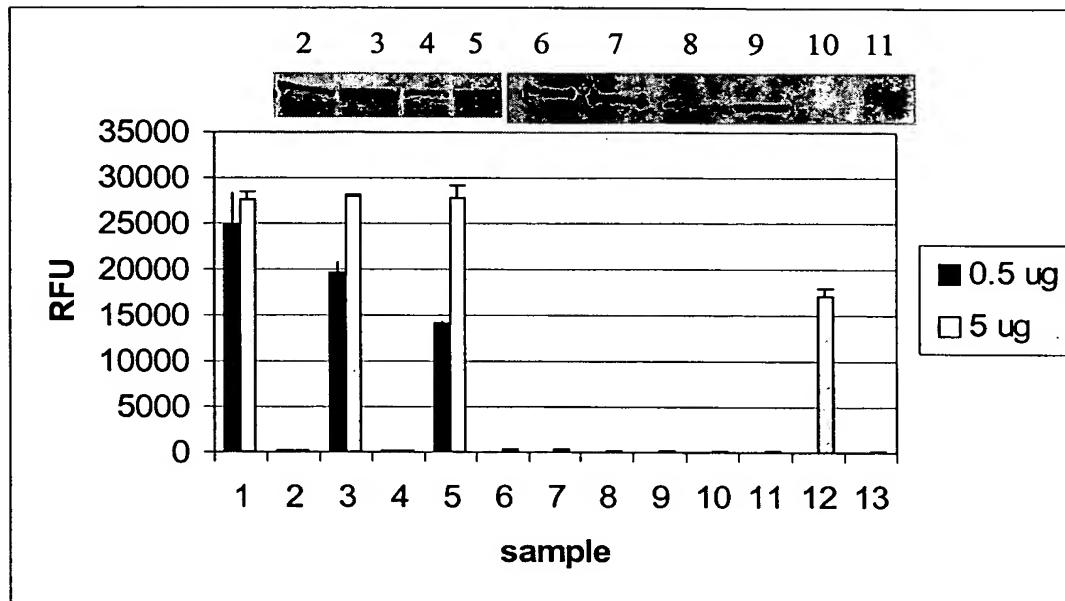


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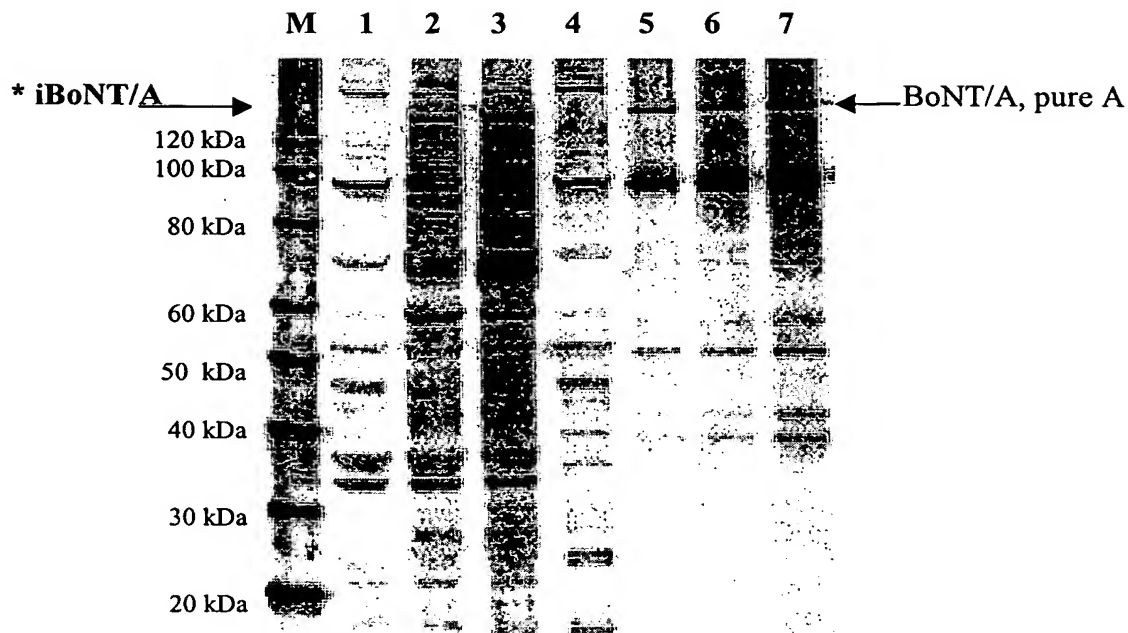


1. LCA in pBAC-1, 11/02
2. LCA-mutant in pBAC-1
3. LCA-wt in pBAC-1
4. LCA-mutant in pBACgus-1
5. LCA-wt in pBACgus-1
6. iBoNT/A in pBAC-1, 0.5 μ g
7. iBoNT/A in pBACgus-1, 0.5 μ g
8. iBoNT/A in pBAC-1, 1 μ g
9. iBoNT/A in pBACgus-1, 1 μ g
10. AcNPV only, negative control
11. Uninfected Sf21
12. E.Coli expressed rLCA
13. Substrate

Figure 10

```
1      MPFVNKQFNY KDPVNGVDIA YIKIPNAGQM QPVKAFKIHN KIWVIPERDT
51     FTNPEEGDLN PPPEAKQVPV SYDSTYLST DNEKDNYLKG VTKLFERIYS
101    TDLGRMLLTS IVRGIPFWGG STIDTELKVI DTNCINVIQP DGSYRSEELN
151    LVIIGPSADI IQFECKSFGH EVLNLTRNGY GSTQYIRFSP DFTFGFEESL
201    EVDTNPLLGA GKFDTPPAVT LAHELIYAGH RLYGIAINPN RVFKVNTNAY
251    YEMSGLEVSF EELRTFGGHD AKFIDSLQEN EFRLYYYNKF KDIASLTNKA
301    KSIVGTTASL QYMKNVFKEK YLLSEDTSGK FSVDKLKFDK LYKMLTEIYT
351    EDNFVKFFKV LNRKTYLNFD KAVFKINIVP KVNXYTIYDGF NLRNTNLAAN
401    FNGQNTENN MNFTKLKNFT GLFEFYKLLC VRGIITSKTK SLDKGYNKAL
451    NDLCIKVNNW DLFFSPSEDN FTNDLNKGEE ITSDTNIEAA EENISLDLIQ
501    QYLTTFNFDN EPENISIENL SSDIIGQLEL MPNIERFPNG KKYELDKYTM
551    FHYLRAQEFE HGKSRIALTN SVNEALLNPS RVYTFSSDY VKKVNKATEA
601    AMFLGWVEQL VYDFTDETSE VSTTDKIADI TIIIPYIGPA LNIGNMLYKD
651    DFLGALIFSG AVILLEFIPE IAIPVLGTFA LVSYIANKVL TVQTIDNALS
701    KRNEKWDEVY KYIVTNWLAK VNTQIDLIRK KMKEALENQA EATKAIINYQ
751    YNQYTEEEKN NINFNIDDL SSKNESINKA MININKFLNQ CSVSYLMNSM
801    IPYGVKRLED FDASLKDALL KYIYDNRGTL IGQVDRCLKD VNNLTSTDIP
851    FQLSKYVDNQ RLLSTFTEYI KNIINTSILN LRYESNHLID LSRVASKINI
901    GSKVNFDPID KNQIQLFNLE SSKIEVILKN AIVYNSMYEN FSTSFWIRIP
951    KYFNSISLNN EYTIINCME NSGWKVSLEY GEIWTQLQDT QEIKQRVVFV
1001   YSQMINISDY INRWIFVTIT NNRLNNSKIY INGRLLIDQKP ISNLGNIHAS
1051   NNIMFKLDGC RDTHRYIWIW YFNLFDKELN EKEIKDLYDN QSNSGILKDF
1101   WGDYLYQYDKP YYMLNLYDPN KYVDVNVVGI RGYMYLKGPR GSVMTTNIYL
1151   NSSLYRGTKF IIKKYASGNK DNIVRNNDRV YINVVVKKE YRLATNASQA
1201   GVEKILSALE IPDVGNLSQV VVMKSKNDQG ITNKCKMNLQ DNNGNDIGFI
1251   GFHQFNNAIAK LVASNWNRYQ IERSSRTLGC SWEFIPVDDG WGERPLHHHH
1301   HH
```

Figure 11.



M, MagicMark

1, AcNPV, negative control

2, pBAC-1/iBoNT/A (H227Y)-His6

3, pBACgus-1/iBoNT/A (H227Y)-His6

4, uninfected Sf21 cells

5, Native BoNT/A, Pure A, 10 ng

6, Native BoNT/A, Pure A, 20 ng

7, Native BoNT/A, Pure A, 50 ng